Transpl Int (1999) 12: 244–249

ORIGINAL ARTICLE

Simone D. P. Vannini
Brunello L. Mazzola
Luca Rodoni
Anita C. Truttmann
Bendicht Wermuth
Mario G. Bianchetti
Paolo Ferrari

Permanently reduced plasma ionized magnesium among renal transplant recipients on cyclosporine

Received: 28 May 1998
Received after revision: 27 November 1998
Accepted: 18 December 1998

S. D. P. Vannini · B. L. Mazzola · L. Rodoni · A. C. Truttmann · M. G. Bianchetti
Division of Pediatric Nephrology, Department of Pediatrics, University of Berne, CH-3010 Berne, Switzerland

S. D. P. Vannini · B. L. Mazzola · L. Rodoni · P. Ferrari
Division of Nephrology, Department of Internal Medicine, University of Berne, CH-3010 Berne, Switzerland

B. Wermuth
Department of Clinical Chemistry, University of Berne, CH-3010 Berne, Switzerland

M. G. Bianchetti (✉)
Division of Pediatric Nephrology, University Children's Hospital, Inselspital, CH-3010 Berne, Switzerland
e-mail: mbianche@insel.ch
Fax: + 41-3-632-94-44

Abstract Hypomagnesemia is common after kidney transplantation. Until recently, only the determination of total plasma magnesium was possible, whereas the assessment of ionized magnesium has since become practicable. One hundred and nine renal transplant patients on cyclosporine with allografts functioning stably for more than 6 months and plasma creatinine levels of less than 200 μmol/l entered the study. Total and ionized circulating magnesium were assessed among these 109 patients, as well as among 15 renal transplant patients not on cyclosporine and 21 healthy volunteers. Cyclosporine patients showed significantly lower total and ionized circulating magnesium values than the two control groups. Plasma total and ionized magnesium levels were also significantly lower among cyclosporine patients treated concurrently with insulin or oral hypoglycemic agents than among those who were not. No correlation was noted between time after transplantation and plasma magnesium with respect to patients on cyclosporine. In conclusion, the study demonstrates that a large subset of renal transplant patients treated with cyclosporine have permanent deficiencies of ionized and total magnesium. The tendency towards hypomagnesemia is also more pronounced among patients with diabetes mellitus.

Key words Kidney transplantation, magnesium, cyclosporine · Magnesium, cyclosporine, kidney transplantation · Cyclosporine, kidney transplantation, magnesium

Introduction

Hypomagnesemia is often encountered early after kidney transplantation among subjects treated with cyclosporine [5, 11]. However, some observations suggest that permanent magnesium depletion subsists after renal transplantation [3]. Circulating magnesium exists in a dissociated (ionized) and in an undissociated form [17, 23]. Until recently, only the determination of total magnesium was possible in clinical practice. Now, the assessment of circulating dissociated magnesium – the most attractive fraction with respect to physiological and biological effects – has become practicable [6, 15, 19, 23]. Due to kidney disease, the proportion of ionized to total circulating magnesium is sometimes altered [14, 20, 22]. It was therefore tempting to speculate on an altered ratio of dissociated to undissociated circulating magnesium after renal transplantation as well. Total and dissociated circulating magnesium were therefore assessed among renal transplant recipients, both on cyclosporine and not, and healthy subjects.

Patients and methods

Eligible for the study were renal transplant recipients on cyclosporine – with allografts functioning stably for more than 6 months, plasma creatinine levels of less than 200 μmol/l, and normal blood pressure – who were undergoing regular follow-up at the Division of Nephrology, Department of Internal Medicine, or at the Division of Pediatric Nephrology, Department of Pediatrics, University of Berne, Switzerland. One hundred and nine patients (58 female and 51 male, ranging in age from 7 to 74, a median of 48 years) entered the study between January and March of 1998. Apart from cyclosporine (Sandimmun Neoral) b.i.d., the medications used for these patients are provided in Table 1.

In the case of this group, sitting blood pressure (the first and fifth sound) and heart rate were measured and venous blood specimens drawn anaerobically and without stasis in the morning, after approximately 10–12 h of fasting, to determine packed cell volume, whole blood cyclosporine, plasma creatinine, urea, uric acid, sodium, potassium, total magnesium, ionized calcium and magnesium, blood pH, and carbon dioxide pressure. The protocol, with the exception of the determination of cyclosporine, was also applied to two control groups consisting of 15 patients (10 women and 5 males, ranging in age from 41 to 74, a median of 62 years) who had undergone renal transplantation before 1986 and had never been on cyclosporine, and 21 healthy volunteers (11 men and 10 women, ranging in age from 23 to 48, a median of 27 years), respectively. The medications used for the 15 patients not treated with cyclosporine are given in Table 1.

The study had been authorized by the Hospital's Ethical Committee, and informed consent was obtained from the participants.

All measurements were performed in duplicate. Packed cell volume was assessed by means of a microhematocrit centrifuge. The whole blood cyclosporine trough level was measured using a specific monoclonal fluorescent polarization immunoassay [24]. Creatinine (kinetic alkaline picrate method), albumin (bromcresol purple method), urea (urease method), uric acid (uricase method) and total magnesium (xylidil blue method) [12] were measured colorimetrically with the help of a Hitachi automatic clinical analyzer. Direct ion-selective electrodes were used for the measurement of pH, carbon dioxide pressure, sodium, potassium, and ionized calcium and magnesium. Plasma magnesium ion values were determined by means of a magnesium electrode, which contains a neutral carrier-based membrane (ETH 7025) incorporated into a standard AVL electrode body by solvent casting (AVL 988-4/Mg Analyzer). This electrode has been characterized recently by our [6, 22] and other laboratories [15, 19].

The ionized magnesium and calcium circulating fractions were calculated by dividing the ionized amount by the corresponding total concentration [6]. Plasma bicarbonate concentration was determined using the Henderson-Hasselbalch equation. The two-tailed Kruskal-Wallis test (a nonparametric analysis of variance for independent samples) with the Bonferroni adjustment, as well as simple regressions with the nonparametric coefficient of determination r^2_s were used for analysis [7]. Significance was assumed at a P value of less than 0.05. The results are given either as median and interquartile ranges or as "box and whisker plots" (boxes are median and interquartile ranges, vertical lines are ranges) [25].

Table 1 Medication other than cyclosporine b.i.d. administered to 109 renal transplant patients treated with and to the control group of 15 renal transplant patients treated without cyclosporine

	Patients treated with cyclosporine	Patients treated without cyclosporine
Immunosuppressive agents[a]	99	15
Azathioprine	53	15
Mofetil mycophenolate	6	–
Prednisone	90[b]	15[c]
Cardiovascular drugs[a]	84	12
Calcium-channel blockers	57	6
β-adrenergic antagonists	67	8
Converting enzyme inhibitors	38	3
Diuretics[d]	44[d]	4[e]
Antidiabetic agents	14	–
Insulin	12	–
Sulfonylureas	2	–
Lipid-lowering drugs	17	3
Sedatives	15	4
Magnesium salts	5	1

[a] Two agents were used simultaneously for some patients (this fact accounts for the apparent mathematical discrepancies)
[b] Dose ranging from 0.02 to 0.30 (median 0.10) mg/kg daily
[c] Dose ranging from 0.12 to 0.22 (median 0.15) mg/kg daily
[d] Either benzothiadiazines and related agents (n = 30) or high-ceiling diuretics (n = 14)
[e] Benzothiadiazines

Results

Table 2 provides information on clinical and biochemical findings for the group of renal transplant patients treated with cyclosporine and for the two control groups. The group of cyclosporine-treated patients was studied from 0.8 to 18 years (a median of 6.0 years) after transplantation, the control group of transplant patients not receiving cyclosporine, from 13 to 20 years (a median of 16 years) after transplantation ($P < 0.05$).

Body height and plasma albumin levels were significantly lower and age, blood pressure, plasma creatinine, urea, uric acid, and potassium higher among renal transplant patients than among healthy control subjects. Body weight, heart rate, plasma sodium, plasma ionized calcium, and the acid base balance did not differ statistically between patients treated with cyclosporine and healthy controls. Body weight was lower and blood pressure, plasma creatinine, urea, and uric acid higher among patients treated with than among those not receiving cyclosporine. Body height, heart rate, plasma sodium, potassium, ionized calcium, the acid base balance, and plasma albumin were similar among patients tretaed with and without cyclosporine.

Total and ionized plasma magnesium levels were significantly lower among patients treated with cyclosporine [0.74 mmol/l (0.69–0.78 mmol/l) and 0.49 mmol/l (0.46–0.53 mmol/l), respectively] than among patients not receiving cyclosporine [0.80 mmol/l (0.77–0.86 mmol/l), and 0.53 mmol/l (0.52–0.57 mmol/l)] and healthy controls [0.81 mmol/l (0.78–0.87 mmol/l) and 0.53 mmol/l (0.52–0.58 mmol/l)], as given in Fig. 1.

Table 2 Clinical and biochemical findings for renal transplant patients treated with cyclosporine and for the two control groups. Results are given as median and interquartile ranges

	Renal transplant patients treated with cyclosporine	Control groups	
		Renal transplant patients treated without cyclosporine	Healthy subjects
n	109	15	21
Sex, female/male	58 / 51	10 / 5	10 / 11
Age, years	48[1] (36–57)	61 (53–67)	27 (25–29)
Time since transplantation, years	6.0[5] (3.4–10)	16 (15–17)	–
Body weight, kg	64 (56–79)	71 (63–80)	63 (60–70)
Height, m	1.62[2] (1.57–1.68)	1.60 (1.55–1.65)	1.74 (1.71–1.80)
Blood pressure, mm Hg	130[2,5] (125–140)/85[2] (79–90)	140 (126–144)/88 (81–90)	116 (105–126)/ 74 (70–79)
Heart rate, per min	66 (60–72)	66 (63–71)	62 (55–68)
Packed cell volume	0.40[3] (0.36–0.42)	0.44 (0.41–0.45)	0.43 (0.41–0.45)
Cyclosporine dosage, mg/kg daily	2.9 (2.1–4.1)	–	–
Whole blood cyclosporine concentration, μg/l	134 (112–149)	–	–
Plasma creatinine, μmol/l	126[3] (109–141)	98 (96–108)	97 (92–102)
Plasma urea, mmol/l	9.8[3] (7.3–12.5)	7.5 (6.4–7.9)	5.2 (4.9–6.6)
Plasma uric acid, μmol/l	417[4] (329–494)	351 (285–390)	270 (255–312)
Plasma sodium, mmol/l	141 (139–142)	141 (140–143)	140 (138–141)
Plasma potassium, mmol/l	4.16[4] (3.89–4.55)	3.95 (3.70–4.18)	3.89 (3.73–4.00)
Plasma ionized calcium, mmol/l	1.28 (1.25–1.36)	1.28 (1.25–1.35)	1.27 (1.25–1.30)
Blood pH	7.35 (7.34–7.38)	7.36 (7.33–7.41)	7.36 (7.34–7.39)
Blood carbon dioxide pressure, mm Hg	5.96 (5.32–6.56)	6.04 (5.60–6.51)	6.19 (5.99–6.47)
Plasma bicarbonate, mmol/l	24.5 (22.1–26.3)	24.9 (21.5–29.0)	25.4 (23.5–27.2)
Plasma albumin, g/l	38[2] (36–40)	38 (36–39)	43 (41–45)

[1] $P < 0.01$ and [2] $P < 0.05$ versus healthy subjects; [3] $P < 0.01$ and [4] $P < 0.05$ versus renal transplant patients without cyclosporine and healthy subjects; [5] $P < 0.05$ versus renal transplant patients without cyclosporine

Table 3 Influence of treatment with diuretics, cardiovascular drugs, and insulin or oral hypoglycemic agents on circulating magnesium among 109 renal transplant patients treated with cyclosporine

	Patients treated with			Patients treated without		
	n	Plasma total magnesium mmol/l	Plasma ionized magnesium mmol/l	*n*	Plasma total magnesium mmol/l	Plasma ionized magnesium mmol/l
Antidiabetic agents	14	0.70* (0.65–0.73)	0.47* (0.43–0.50)	95	0.74 (0.70–0.80)	0.50 (0.47–0.55)
Diuretics	44	0.75 (0.65–0.82)	0.49 (0.44–0.53)	65	0.74 (0.70–0.78)	0.50 (0.47–0.55)
Cardiovascular drugs	84	0.74 (0.69–0.80)	0.50 (0.45–0.54)	25	0.75 (0.73–0.77)	0.49 (0.44–0.51)
Diuretic and antidiabetic agents[a]	53	0.74 (0.65–0.81)	0.49 (0.45–0.53)	56	0.74 (0.70–0.78)	0.50 (0.46–0.54)

[a] Insulin or sulfonylureas

* $P < 0.02$ versus patients treated without sulfonylureas or insulin

The plasma ionized magnesium fraction among patients treated with [0.67 (0.65–0.70)] and without [0.66 (0.64–0.68)] cyclosporine was similar to that in the group of healthy controls [0.67 (0.65–0.70)].

Plasma total and ionized magnesium values were both significantly lower in the subgroup of cyclosporine patients treated with antidiabetic agents than among the remainder of patients receiving cyclosporine (Table 3). However, plasma magnesium values in the subgroups of cyclosporine patients treated with diuretics or cardiovascular drugs were similar to those among the remainder of patients receiving cyclosporine. The same also applies to the cumulated subgroup of cyclosporine patients treated with both antidiabetic agents and diuretics. The 56 cyclosporine-treated patients not receiving antidiabetic agents or diuretics exhibited significantly lower ($P < 0.005$) plasma total [0.74 mmol/l (0.70–0.78 mmol/l)] and ionized [0.50 mmol/l (0.46–0.54 mmol/l)] magnesium levels than the control group of healthy subjects [0.81 mmol/l (0.78–0.89 mmol/l) and 0.54 mmol/l (0.53–0.59 mmol/l), respectively].

With respect to patients treated with cyclosporine, no significant correlation was noted between time since transplantation, cyclosporine dosage, whole blood cyclosporine trough level, or plasma creatinine, taken as

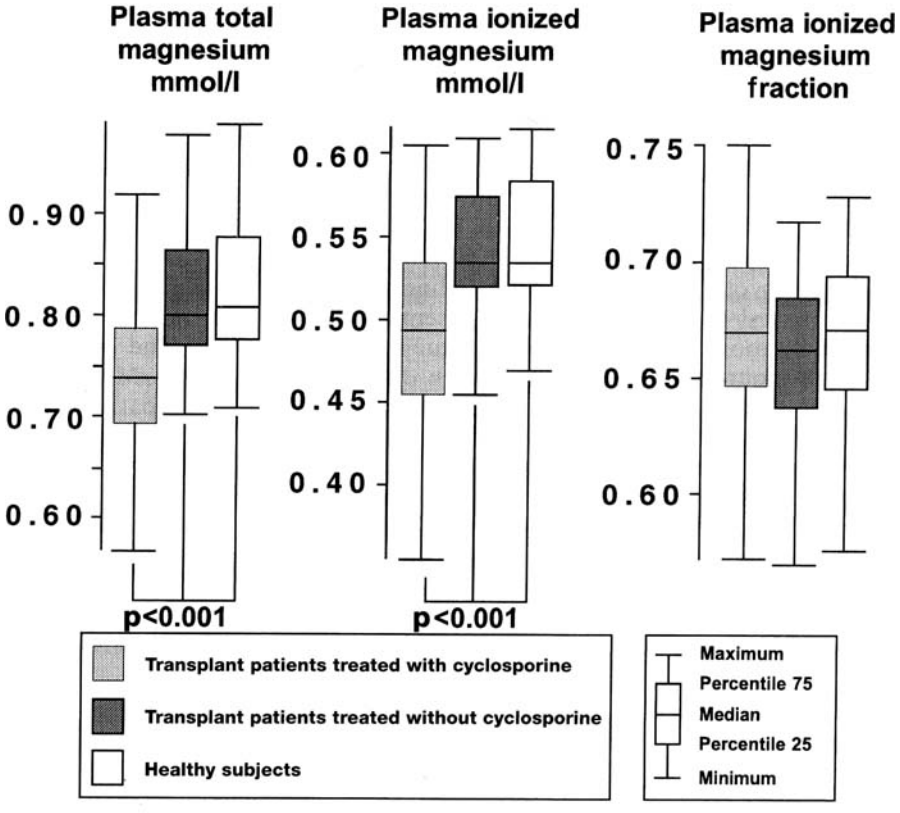

Fig. 1 Plasma total magnesium, plasma ionized magnesium and plasma ionized magnesium fraction among transplant patients treated with cyclosporine, transplant patients treated without cyclosporine, and healthy subjects. The results are given as "box and whisker plots": *boxes* are median and interquartile ranges, *vertical lines* are ranges

independent values, and plasma total or ionized magnesium, taken as dependent values (Table 4).

Discussion

In renal transplantation, cyclosporine has been repeatedly reported to cause low total plasma magnesium values during the early post-transplant period [3, 5, 11]. The present study demonstrates that a large subset of renal transplant patients treated with cyclosporine have profound and permanent deficiencies of both ionized and total magnesium that cannot be explained on the basis of the simultaneous use of diuretics. Apart from that, the study indicates that the tendency towards hypomagnesemia is more pronounced among patients with diabetes mellitus, a well-recognized condition that is linked with magnesium deficiency.

The median age within the group of healthy subjects included in this study was lower than within the groups of patients treated with and without cyclosporine. In the case of healthy subjects, however, circulating magnesium does not vary between the ages of 10 and 75 years [26]. Plasma creatinine was slightly higher among cyclosporine patients than among those not receiving it and the control subjects, suggesting a link between the tendency towards hypomagnesemia and that towards hypercreatininemia noted in cyclosporine patients. Since a moderate reduction of renal function

Table 4 Regression analysis for 109 renal transplant patients. None of the regressions were found to be significant

Dependent value	Independent value	r^2s
Plasma ionized magnesium, mmol/l	Time since transplantation, years	0.0041
Plasma total magnesium, mmol/l	Time since transplantation, years	0.0013
Plasma ionized magnesium, mmol/l	Cyclosporine dose, mg/kg daily	0.0155
Plasma total magnesium, mmol/l	Cyclosporine dose, mg/kg daily	0.0004
Plasma ionized magnesium, mmol/l	Whole blood cyclosporine level, µg/l	0.0001
Plasma total magnesium, mmol/l	Whole blood cyclosporine level, µg/l	0.0029
Plasma ionized magnesium, mmol/l	Plasma creatinine, µmol/l	0.027
Plasma total magnesium, mmol/l	Plasma creatinine, µmol/l	0.069

slightly reduces magnesium excretion [8], this suggestion does not apply to our patients.

The present study did not specifically address the mechanism underlying hypomagnesemia following renal transplantation. A large body of evidence indicates that hypomagnesemia after cyclosporine treatment is caused by enhanced urinary excretion [5, 18] and perhaps even by intracellular uptake of the ion [18]. Apart from cyclosporine, diabetes mellitus [10, 16] and the use of benzothiadiazines or high-ceiling diuretics [9] are expected to enhance hypomagnesemia after renal transplantation. In this study, hypomagnesemia was more pronounced among patients with diabetes mellitus. In contrast to this, plasma magnesium values were similar among patients treated with and without diuretics. Circulating magnesium is present in three different states: dissociated (ionized); bound to albumin; and complexed to phosphate, citrate, and other anions. Because albumin-bound and complexed magnesium are unavailable for biochemical processes, only ionized magnesium shows biological activity. The measurement of total circulating magnesium is therefore an inaccurate guide to the biologically active circulating fraction [17, 23]. For the past 20 years, the Swiss Center for Chemical Sensors has synthesized ionophores and placed them in appropriate membranes that are now sufficiently selective to determine dissociated magnesium [21]. In the present study, the key to a better understanding of the extracellular magnesium metabolism among renal transplant patients who were given cyclosporine was that both total and dissociated magnesium were reduced, as previously noted in two studies performed with very few patients [4, 13].

The present observations have important implications. Magnesium depletion has been implicated in various pathological conditions, such as vasoconstriction, arterial hypertension, cardiac arrhythmias, thrombosis and atherosclerosis; all the above occurring to an increased extent among renal transplant recipients [1, 2]. In view of these possible adverse reactions to hypomagnesemia, it behooves us to alert to the occurrence of permanent hypomagnesemia among renal transplant patients receiving cyclosporine so that affected subjects can be given a prompt replacement.

References

1. Abbott LG, Rude RK (1993) Clinical manifestations of magnesium deficiency. Miner Electrolyte Metab 19: 314–322
2. Al-Ghamdi SM, Cameron EC, Sutton RA (1994) Magnesium deficiency: pathophysiologic and clinical overview. Am J Kidney Dis 24: 737–752
3. Al-Khursany I, Thomas TH, Harrison K, Wilkinson R (1992) Reduced erythrocyte and leukocyte magnesium is associated with cyclosporin treatment and hypertension in renal transplant patients. Nephrol Dial Transplant 7: 251–255
4. Allegra A, Corica F, Ientile R, Corsonello A, Sparacino V, Accettola F, Caputo F, Macaione S, Buemi M (1998) Plasma (total and ionized), erythrocyte and platelet magnesium levels in renal transplant recipients during cyclosporine and/or azathioprine treatment. Magnes Res 11: 11–18
5. Barton CH, Vaziri ND, Martin DC, Choi S, Alikhani S (1986) Hypomagnesemia and renal magnesium wasting in renal transplant recipients receiving cyclosporine. Am J Med 83: 693–699
6. Blumberg D, Bonetti A, Jacomella V, Capillo S, Truttmann AC, Lüthy CM, Colombo JP, Bianchetti MG (1998) Free circulating magnesium and renal magnesium handling during acute metabolic acidosis in humans. Am J Nephrol 18: 233–236
7. Brown GW, Hayden GF (1985) Nonparametric methods – clinical application. Clin Pediatr 24: 490–498
8. Coburn JW, Popovtzer MM, Massry SG, Kleeman CR (1969) The physiochemical state and renal handling of divalent ions in chronic renal failure. Arch Intern Med 124: 302–311
9. Davies DL, Fraser R (1993) Do diuretics cause magnesium deficiency? Br J Clin Pharmacol 36: 1–10
10. Husmann MJW, Fuchs P, Truttmann AC, Laux-End R, Mullis PE, Peheim E, Bianchetti MG (1997) Extracellular magnesium depletion in pediatric patients with insulin-dependent diabetes mellitus. Miner Electrolyte Metab 23: 121–124
11. Kim HJ, Ahn YH, Kee CS, Lee KS, Kwak JY (1994) Early short-term profile of serum magnesium in living donor renal transplant recipients on cyclosporine. Transplant Proc 26: 2178–2180
12. Mann CK, Yoe JH (1956) Spectrophotometric determination of magnesium with sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphtalene-1'-(2-hydroxybenzen-5-sulfonate). Anal Chem 28: 202–205
13. Markell MS, Altura BT, Sarn Y, Barbour R, Friedman EA, Altura BM (1993) Relationship between ionized magnesium and cyclosporine level in renal transplant recipients. Ann N Y Acad Sci 696: 408–411
14. Markell MS, Altura BT, Sarn Y, Delano BG, Ifudu O, Friedman EA, Altura BM (1993) Deficiency of serum ionized magnesium in patients receiving hemodialysis or peritoneal dialysis. ASAIO J 39: 801–804
15. Marsoner HJ, Spichiger UE, Ritter C, Sachs C, Ghahramani M, Offenbacher H, Kroneis H, Kindermans C, Dechaux M (1994) Measurement of ionized magnesium with neutral carrier based ISE's. Progress and results with the AVL 988–4 magnesium analyzer. Scand J Clin Lab Invest Suppl 217: 45–51
16. Mullis P, Bianchetti MG (1998) High-fiber diets may be responsible for hypomagnesaemia in diabetic patients. Nephron 78: 238–239
17. Nadler JL, Rude RK (1995) Disorders of magnesium metabolism. Endocrinol Metab Clin North Am 24: 623–641

18. Rahman MA, Ing TS (1989) Cyclosporine and magnesium metabolism. J Lab Clin Med 114: 213–214
19. Rouilly M, Rusterholz B, Spichiger UE, Simon W (1990) Neutral ionophore-based selective electrode for assaying the activity of magnesium in undiluted blood serum. Clin Chem 36: 466–469
20. Saha H, Harmoinen A, Pietilä K, Mörsky P, Pasternack A (1996) Measurement of serum ionized versus total levels of magnesium and calcium in hemodialysis patients. Clin Nephrol 46: 326–331
21. Spichiger UE (1993) History and development of magnesium-selective ionophores and magnesium-selective electrodes. Electroanalysis 5: 739–745
22. Truttmann AC, Laux-End R, McGuigan JAS, Bianchetti MG (1995) Plasma-ionized magnesium concentration in a child with magnesium-losing tubulopathy. Nephron 71: 238–239
23. Truttmann AC, Bettinelli A, Bianchetti MG (1997) Métabolisme du magnésium pour le clinicien: une mise au point actuelle et simple. Méd Hyg Geneve 55: 551–553
24. Wang P, Meucci V, Simpson E, Morrison M, Lunetta S, Zajac M, Boeckx R (1990) A monoclonal antibody fluorescent polarization imunoasssay for cyclosporine. Transplant Proc 22: 1186–1188
25. Williamson DF, Parker RA, Kendrick JS (1989) The box plot: a simple visual method to interpret data. Ann Intern Med 110: 916–921
26. Yang XY, Hosseini JM, Ruddel ME, Elin RJ (1990) Blood magnesium parameters do not differ with age. J Am Coll Nutr 9: 308–313